Attorney's Docket No.: 14176-003001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Dr. Chau-Ting Yeh
Serial No. : 10/730,632
Filed : December 8, 2003
Title : NOVEL VIRAL SEQUENCES

Art Unit : 1632
Examiner : David A. Montanari

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION BY DR. CHAU-TING YEH UNDER 37 C.F.R. 1.132

I, Chau-Ting Yeh, hereby declare that:

1. I am the sole inventor of the subject matter described and claimed in the above-identified application, which relates to novel viral sequences, including NV-F (SEQ ID NO: 1) shown at page 1 of the application.

2. I supervised the following three experiments:

Experiment 1

The protein encoded by NV-F was expressed and used to generate antibodies according to methods described at page 5, line 28 to page 6, line 13 of the application. The coding sequence of NV-F was inserted into a pYES2/NT vector (Invitrogen Corporation, Carlsbad, CA) so that it was fused in-frame with both the polyhistidine coding region and the Xpress coding sequence in this vector. The resulting fusion coding region was then released by restriction

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

December 21, 2005
Date of Deposit

Lisa G. Gray
Signature

Lisa G. Gray
Typed or Printed Name of Person Signing Certificate

enzyme digestion, blunt-ended, and inserted into a pBacPAK8 vector (Clontech Laboratories, Inc., Palo Alto, CA) to generate an expression vector. This vector was expressed in insect cells using the BacPak Baculovirus Expression System (Clontech Laboratories) by a standard method. Expressed polyhistidine-NV-F-Xpress fusion protein was purified by a Ni2+-charged affinity column and subjected to gel electrophoresis-Coomassie blue staining analysis. The results are shown in Fig. 1A, left gel below. It was found that the fusion protein was expressed in the insect cells and purified from insect cell lysate. See lane 1 for purified protein and lane 2 for total cell lysate.

The purified fusion protein was then used to develop mouse polyclonal antibody against NV-F by a standard method. Western blot was conducted using the antibody. It was found that the antibody recognized the purified fusion protein and a corresponding band in the insect cell lysate. See Fig. 1A, right gel, lanes 1' and 2'.

Western blot analysis was also conducted on NV-F protein that was not fused to any heterologous protein using the mouse anti-NV-F antibody, a pre-immune mouse serum, and a serum from a non-A-to-E-hepatitis patient. Referring to Fig. 1B below, the results show that the NV-F protein was recognized by the mouse anti-NV-F antibody (lane 1) and the patient's serum (lane 3), but not by the pre-immune serum (lane 2).

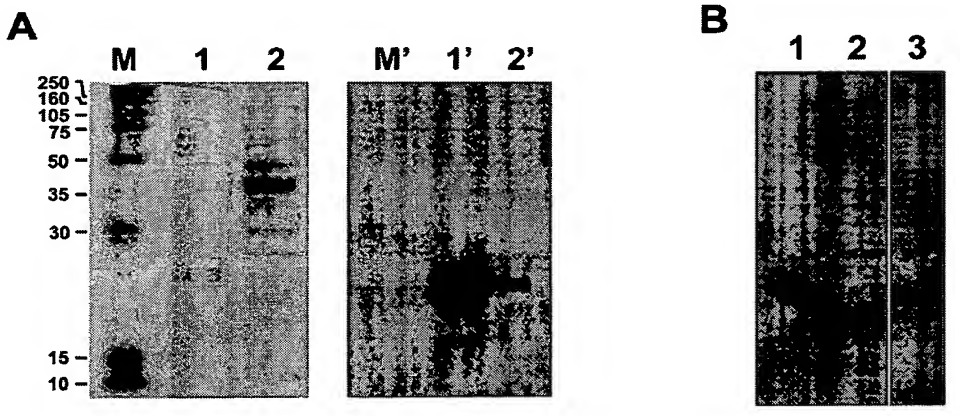

Fig. 1

Experiment 2

Immunofluorescence analysis was performed on liver samples from the same patient. Fragments of liver specimens were snap frozen in isopentane cooled with liquid nitrogen and stored at –70°C. Cryostat sections, 5 μm in thickness, were obtained, dried overnight at room temperature, and fixed in acetone at 0°C for 5 minutes. Immunofluorescence staining was performed using the mouse anti-NV-F antibody followed by incubation of FITC conjugated rabbit anti-mouse antibody (Jackson Immuno Research Laboratories, West Grove, Pennsylvania). The sections were then stained with DAPI (200 ng/ml) to visualize nuclei before examination by confocal microscopy the Leica TCS SP2 Laser Scanning Spectral Confocal System. As shown in Fig. 2 below, NV-F protein was detected in the cytoplasm of the patient's hepatocytes either in a speckle or a homogenous pattern. The upper left panel, upper right panel, and lower left panel respectively show the positive results of staining FITC-anti-NV-F, DAPI, and both; the lower right panel shows the negative result of staining by a negative control pre-immune serum. The analysis was repeated on a chronic hepatitis B patient, who was seropositive for NV-F. A similar staining pattern was observed.

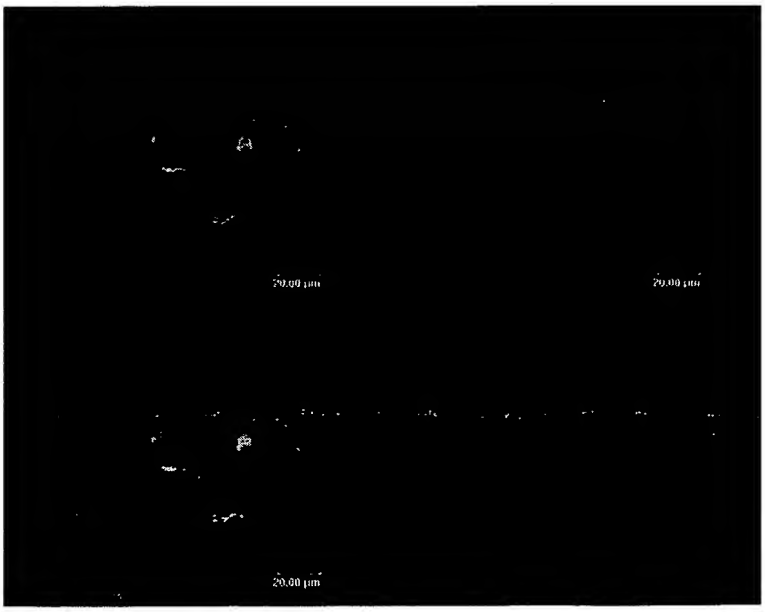

Fig. 2

Experiment 3

The clinical course of a patient having fulminant non-A-to-E hepatitis was examined and summarized in Fig. 3 below. The solid line and gray line represent ALT level (U/L) and Bilirubin level (mg/dL), respectively. NV-F sequence was detected ("+") during the hepatitis flare. These results show that the patient's serum was positive for NV-F sequence during the period of hepatitis but was negative after recovery. In other words, they demonstrate that nucleotide sequence of NV-F encodes a protein that correlates with hepatitis.

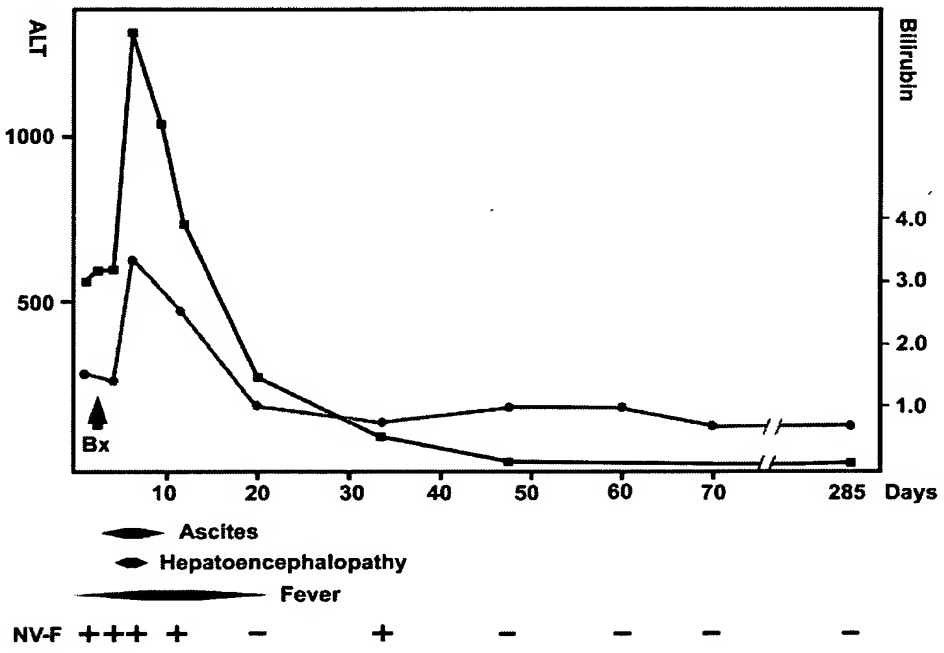

Fig. 3

3. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that

such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully Submitted,

Date: 2005. 11. 29

Chau-Ting Yeh, Ph. D.
Liver Research Unit
Chang Gung Memorial Hospital
199 Tung Hwa North Road
Taipei, Taiwan